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THE EFFECT OF PH ON THE CILIARY ACTIVITY OF HUMAN RESPIRATORY EPITHELIUM IN TISSUE CULTURE

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INTRODUCTION

Cilia were first seen in 1675 by Leeuwenhoek and were first described in 1684 by de Heide.[42] Since that time they have been found in a variety of animals and in human beings they have been found in the central canal of the spinal cord and ventricles of the brain as well as in the respiratory and reproductive systems.[36] In the respiratory tract ciliated cells are found from the nose and accessory sinuses down to the bronchioles of 0.4 mm diameter.[28] In the human trachea the ciliated epithelium is of the pseudostratified columnar type (Fig. 1). It is composed of four types of cells, the basal cells, the intermediate cells, the mucus-producing cells, and the ciliated cells, situated upon a basement membrane and a dense network of fine collagenous and reticular fibrils with occasional nerves penetrating the basement membrane.[44] (Fig. 2) Most of the respiratory tract is lined with similar epithelium although in the accessory sinuses the ciliated epithelium is composed of one layer of replacement cells and one layer of short, almost cuboidal cells.[20] Nasal polyps also have a slightly different epithelial covering in that there is no consistent basement membrane.[45] Inflammatory changes are also present in nasal polyp epithelium.[35]

The cilia themselves are structures which have a pendular movement and are able to move water and particles at right angles to their length. There are, on the average, 200 cilia on each ciliated cell from the human trachea. Each cilium has an average

length of 6μ and an average diameter of 0.3μ. It is composed of two central filaments and nine peripheral filaments all surrounded by a membrane which is continuous with the cell membrane. The two central filaments stop at the cell surface while the nine peripheral filaments continue into the basal body which is located just within the cytoplasm of the cell[44] (Fig. 3).

Ciliary motion consists of an effective stroke and a recovery stroke, the effective stroke being three times as fast as the recovery stroke.[10] The frequency of the beat has been measured in various mammals and found generally to be between 600 and 1500 beats per minute,[10,12,14,27] although Frenckner and Richtner (1940) measured the ciliary beat rate as 160-250 beats per minute in the human nose[16] and Proetz (1953), also studying human nasal cilia, measured the beat frequency as 480-720 beats per minute.[34] Cilia are also co-ordinated, so that two well-defined lines exist in ciliated epithelium: one in which cilia beat together and one in which they beat in succession to give the appearance of waves called "metachronal waves". The metachronal waves result because each cilium of the wave is at a slightly different stage in its beat from its neighbors that lie along the line of wave transmission.[42] There are several types of metachronal waves; the symplectic, in which the metachronal wave travels in the same direction as the effective stroke, the diaplectic, in which the metachronal

wave travels at right angles to the ciliary beat, and the antiplectic metachronal wave which is found in the cilia of the mammalian respiratory tract in which the metachronal wave moves in the opposite direction to the effective stroke.[44] Numerous theories[26,36,42,44] have been proposed for the control of this motion but to date no satisfactory explanation has been given for the control of mammalian cilia.

Many factors influence ciliary activity, the predominant ones being the temperature, humidity, viscosity, hydrostatic pressure, osmotic pressure, oxygen supply and concentrations of various ions (particularly the hydrogen ion) of the surrounding environment.[42] The normal temperature of the inferior turbinate of the human nose has been found by Cone to be 32°C. and other investigators have generally confirmed this although values ranging from 32°C.-36.6°C. have been reported.[34] The temperature of the trachea (as measured in the human or other mammals) is between 32-37°C. and although this range of values is similar to that obtained from the human nose, investigators have found the temperature of the respiratory tract to increase as the alveoli are approached.[10] It has also been observed that the activity of cilia of cells from a single site increases with increasing temperature up to about 40°C. where a decrease in ciliary activity begins to be seen.[10,36] The humidity in the respiratory tract, as reported by most investigators, increases from the nasal cavity (75-95% relative humidity[34]) to the trachea, and there is a further increase in humidity in the alveoli (95-98%

relative humidity)[10]. Dalhamn found that as long as the humidity of the rat trachea was kept at greater than 70% relative humidity there was no harmful drying effect.[10] In my investigation I have studied one of these factors affecting ciliary activity, the pH of the surrounding medium, and have done this through the use of tissue culture of ciliated respiratory epithelium.

Strelin in 1929 was the first investigator to culture ciliated respiratory epithelium successfully. He observed ciliary movement during nine days of tissue culture of rabbit respiratory mucosa.[6] In 1935, Schopper noted ciliary beating in embryonic guinea pig and chick bronchial epithelium through one week of cultivation.[40] In 1939 Proetz and Pfingsten cultured nasal mucosa from full term guinea pig fetuses. They cultured this tissue utilizing a plasma clot technique in hanging-drop slides, the clot being composed of maternal plasma, calcium Ringer's solution and feeding solution.[a] Ciliary activity was present in the tissue and they maintained the explants for three to four weeks.[6,32,33,36] In 1949 Rose, Pomerat and Danes also utilized the plasma clot technique in studying human nasal mucosa in hanging-drop slides, roller tubes and Carrel flasks. The pieces of tissue were placed in a clot composed of 50% cockrel plasma (with penicillin), 25% chick embryo extract and 25% human ascitic fluid. A supernatant nutrient fluid was made up of ascitic fluid, embryo extract and Tyrode's solution.

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[a] Feeding solution contained: peptones, hemin, thyroxin, cystine hydrochloride, insulin, dextrose, sodium and potassium acid phosphates, magnesium Ringer's solution.

Ciliary activity persisted at room temperature for four weeks in a roller tube culture and for eight days in a phase contrast slide.[39] Using similar techniques, Danes also studied pulmonary epithelium of Triturus viridescens and obtained active ciliary motion for as long as six weeks.[6] In 1956 Jordan obtained nasal mucosa at the time of elective operation and, after mincing the tissue, embedded it in plasma clots in roller tubes, and found that the cilia on the mucosal explants continued to beat actively for seven days. No cilia were observed on the outgrowth of new cells, however.[23] Barski in 1957 grew monkey tracheal cells as well as human adult and embryonic respiratory mucosa. He also used the plasma clot technique[b.] and the ciliary activity persisted as long as three weeks in the human tissue and for 45 days in the monkey trachea.[5,6]

Corssen and Allen began their studies using tissue culture in 1958 when they cultured human tracheal and bronchial epithelium obtained while the patients were under general anesthesia. The explants were set up in plasma clots in hanging-drop slides, and ciliary activity reached maximal activity within 2-3 days and was retained about 10-12 days. Rotating globes were found, and the effect of local anesthetics on these rotating globes or "turners" was studied using stoppage of motion of the globes or

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[b.] Barski also used a medium composed of 0.5% lactalbumin hydrolysate in Earle's solution supplemented with 0.1% yeast extract, 5% bovine embryo extract and 10-15% horse serum in addition to the plasma clot.

ciliary arrest as the end point.[7] In 1959 and 1960 they did further studies utilizing these techniques.[8,9] In 1958 Hoch-Ligeti and Hobbs cultured explants of human bronchial epithelium (after they had been trypsinized) in chick plasma clots(chick plasma, chick embryo extract and human serum). They cultured the explants in micro-petri dishes using perforated cellophane disks to support the cultures and developed permanent strains of epithelial cells in which they observed ciliary activity up to the "6th generation" or about 40-50 days.[22] Kellner and Richter[24] and Herrmann[18] also observed ciliary activity in tissue culture of human nasal epithelium in 1958.

Cailleau in 1959 maintained fragments of adult human bronchial tissue in culture which often retained ciliary activity for 60 days or more. The explants were grown on perforated cellophane in medium 199 and 20% human placental serum.[6] Foley established epithelial cell lines from human lung tissue and normal nasal polyps in 1960. He also established cell lines from nasal polyps from children with cystic fibrosis. However, there is no mention of ciliary activity on any of these cells. He used Eagle's medium with a serum supplement of 10% whole pooled human serum.[15]

Ballenger began his studies in 1960 utilizing the method of tissue culture to study the effects of cigarette smoke and its

components on respiratory epithelium.[2] Ballenger's REM (rotating explant method), as he called it, involves obtaining tracheal mucosal scraping from children undergoing tonsillectomy. The tissue is then stored in Gey's Balanced Salt Solution (BSS) (pH 7.2-7.6) with antibiotics until the fragments are placed in plasma clots in Maximow hanging-drop slides. The clots are composed of chick plasma and 50% chick embryo extract. The slides are incubated overnight, and the next day they are observed for "turners". If found, the clot is transferred to a Rose chamber where Gey's BSS pH 7.4 is added to the chamber. The "turners" are then timed to obtain an indication of the baseline speed for each "turner". This is done by clocking the time required for the "turner" to rotate ten revolutions. If this time is constant over a 20-30 minute interval, then the average of these values is the baseline time for ten rotations (an indication of the baseline speed). The "turners" can then be perfused with some substance dissolved in the Gey's BSS to see its effect on the speed of the "turners". The actual rotation of the "turners", which may continue for 4-5 days if left undisturbed in the Maximow slides, represents the co-ordinated metachronal activity of the cilia. If a decrease in rotational speed is noted, this may be due either to a decrease of the frequency of the beat, a decrease of the excursion of the beat, a decreased efficiency of each ciliary cycle, or a loss of the metachronal rhythm.[3,c]

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c. Personal communication, 1965.

The advantages of this method as stated by Ballenger are:

> 1. Temperature and humidity are easily controlled in contrast to methods employing an open trachea or an excised membrane in air.
>
> 2. There is complete avoidance of an indicator which might interact with the cilia, either by reason of weight or its chemical nature as in the particle transport method.
>
> 3. It can be considered, if you will, a "separate, epithelial organ" which is devoid of muscles, a vascular system, and a nervous system.
>
> 4. The rotational activity may be said to reflect the function of an integral part (an extension of the cell membrane) of isolated epithelial cells.
>
> 5. Ability of the cilia to perform this work may be measured quantitatively.[44]

Ballenger claims that the only disadvantages of this method are that the mucous blanket is absent, that it is an in vitro preparation, and that only man and monkeys can be used as sources of tissue.[44]

Kent in 1965 investigated antigen-antibody responses of tissue cultures of small squares of tracheal mucosa from guinea pigs, rabbits and human beings as well as fragments of human nasal and bronchial mucosa. The fragments of ciliated epithelium from the animals and patients were cultured in Leighton tubes or on coverslips in perfusion chambers, the explants being attached to the tube or coverslip by means of a plasma clot or nylon mesh. The cultures were maintained in Eagle's medium with antibiotics and either calf or human sera. They found that the human tracheal tissue obtained by scraping the trachea gave the best growth of

all the human tissue and that the poorest results were obtained from the human nasal specimens.[d] Ciliary activity was present for several weeks once the tissue had been successfully started.

In 1965 Rosan also cultured the epithelium of nasal polyps. He used tissue from polyps of children with cystic fibrosis for the most part, but he also cultured epithelium from "normal" nasal polyps. The tissue was cultured in Carrel flasks utilizing medium 1066 and 10% calf serum. The pH was maintained at approximately 6.9. Ciliary activity was present in tissue from four of the eleven patients with cystic fibrosis and ciliary activity persisted for days or weeks in some parts of the specimens. Although only one control tissue was cultured, no difference could be found when the tissue culture characteristics of the cystic fibrosis tissue were compared to those from non-cystic fibrosis patients.[37]

The latest work has been done by Spock (1966) who has grown rabbit tracheal epithelium as well as human nasal polyp epithelium in Falcon flasks using medium 199 with 10% fetal bovine serum and has studied the effects of the serum of cystic fibrosis patients on ciliary activity.[43]

Various investigators have also studied the effect on ciliary

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d. The poor results obtained with the nasal specimens may have been due to the fact that topical anesthesia was used to remove the specimens. Rose also failed to obtain outgrowth from nasal tissue under these conditions (39).

activity of alterations in the pH of the medium (Table I). The first observations made along these lines were made by Kuhne in 1866 who stated that acids stopped ciliary activity.[36] Since that time investigators have studied the effect of pH on the cilia of various animals. Observations have been made on the *Paramecium* by Chase and Glaser (1930) and by Dryl (1961) who found that the maximum forward movement of the *Paramecium* takes place between a pH of 5.1 and a pH of 6.3.[42]

Work has been done using the gill cilia of the mollusks *Mytilus*, *Mya*, *Pecten*, and *Ostrea* also. Gray (1922, 1928) studied the *Mytilus* gill and found a progressive increase in ciliary motility with an increase in pH from 5.6-9.0.[13] Below pH 5.5-6.0 the cilia will not beat, however, they will continue to beat above pH 9.0.[42] He also found that weak acids (particularly carbonic acid) acted more quickly than the strong mineral acids and attributed this to the fact that weak acids could penetrate the cell more easily than the strong acids.[42] This observation was also substantiated by Haywood(1925), Nomura (1932) and Tomita (1935) who also studied cilia from mollusks as well as by Jucci (1928) who studied cilia on a coelenterate.[42] Tomita (1934) found a steep fall in activity at pH 5.5 with oyster gill cilia.[10]

Studies have also been done using frog pharyngeal epithelium.

Das (1965) found that a decrease of pH to 6.5 excited ciliary motility but that a further decrease below pH 6.0 markedly depressed the cilia. An increase in pH of the solution above 7.5 was always found to be depressant.[13] Seo (1932), on the other hand, studied cila from the frog uterus and observed the length of time required for acid solutions to stop ciliary motion.[41] Both Dad and Seo used HCl to acidify their solutions.

The studies on mammalian cilia have produced varied results. It is generally agreed upon that acids inhibit ciliary activity but responses to alkalinity do not seem to be as dramatic or consistent. Negus (1934) reported that an increase in ciliary activity with dilute alkaline solutions had been found as early as 1907, and he himself found that cilia in extirpated rabbit epithelium would stop beating at a pH less than 6.4 but that they would maintain a vigorous action for 24 hours or more in a physiological saline solution of pH 8.5.[29] Hilding in 1963 reported that an increase in pH to about 9 stimulated the rate of ciliary beat and a decrease to below 6.0 decreased the rate. He felt, however, that in the living person the pH probably did not alter the normal functioning of the cilia.[20]

Paul (1965) states that the average mammalian cell will survive

indefinitely in the range of pH 6.6-7.8 and there are also reports of cells being maintained below 6.6 and above 8.0. In general, however, optimal growth is obtained between pH 7.2 and 7.4, and he advises that the pH of the medium be kept between 6.8 and 7.6.[30] Kent (1965) studied mammalian ciliated epithelial cells in tissue culture and found that pH values below 6.0 and above 8.0 were associated with slowing and cessation of ciliary activity.[25] Kensler and Battista (1966) observed optimal ciliary transport activity on preparations of rabbit tracheal epithelium *in vitro* with pH 6.5-8.5.[44] Spock (1966) has also found that rabbit tracheal ciliated cells in tissue culture would be stopped only when the pH reached 1.0 or 9.0.[43]

The pH of the human nasal mucosa or nasal secretion has been measured by several investigators (Mittermaier(1930), Nungester and Atkinson (1944), Fabricant (1941), Tweedie(1934), Buhrmester (1933,1938), Hilding (1934)[10], Negus (1958)[14]) and the values have ranged between 6.2 and 8.5.

MATERIALS AND METHODS

For the following experiments, nasal polyps and epithelium from the accessory nasal sinuses were obtained from elective operations. After removal, the tissue was placed in a sterile container (petri dish or sputum jar) and kept moist with sterile saline until the following procedure could be carried out. Six petri dishes were then set up with each of the first five dishes containing about 10 ml of Hanks Balanced Salt Solution (BSS)[e], with 50 units of penicillin and 50 µg of streptomycin per milliliter. The sixth petri dish was set up with only one to two ml of Hanks BSS. An additional 5000 units of penicillin and 5000µg of streptomycin were added to the second petri dish and then to the third. The tissue was cleaned with blunt forceps and small scissors during this time and any blood clots removed. The tissue was cleaned further in the fourth and fifth petri dishes if necessary and was then minced up into pieces (explants) approximately 1-2mm square in the sixth dish. This mincing was done with a small curved scissors, taking care to see that the tissue was covered with the top of the petri dish as much as possible during this procedure, and at times required 30 minutes or longer to accomplish. This tissue was left in the Hanks BSS at least one hour before placing the explants in flasks or chambers.

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[e] Microbiological Associates, Bethesda, Maryland.

About ½ ml of BSS containing explants was then placed in each 30 ml Falcon flask with a capillary pipet. Ten to twenty flasks were prepared and 1-2 ml of media[f] (pH 7.2-7.6) were placed in each flask through a millipore filter after which the flasks were placed in the incubator at 37°C. It was also possible to prepare chambers at this time if desired.

The flasks were allowed to stay undisturbed in the incubator for 2-3 days regardless of the pH of the media. At the end of this time the flasks were systematically scanned for "turners" (rotating masses of ciliated epithelial cells)(Figure 4) under x35 magnification. When "turners" were found, they were circled with a wax pencil and the flasks were then taken to the tissue culture room where the "turners" were removed with a capillary pipet. This procedure was carried out by holding the flasks so that the explant (with "turners") to be removed was on the inside of the top surface of the flask. A small amount of media from the flask was taken up in the capillary pipet and then this was injected on the explant. Then the explant was quickly sucked up into the pipet or onto the tip of it and removed from the flask.

The explant was placed on a sterile coverslip (either a 45mm x 50mm coverslip for the Rose chamber(Figure 5) or a 25 mm diameter

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[f] The media is composed of 90% Medium 199(Microbiological Associates) and 10% fetal bovine serum (Microbiological Associates). Penicillin (50 units/ml) and streptomycin (50μg/ml) are also added.

coverslip for a Sykes-Moore chamber(Figure 6) , which had already been placed in a petri dish. The excess media was removed from the explant and one drop of chicken plasma[g] was added followed by one drop of 50% chick embryo extract.[h] These two substances were stirred together and the mixture was allowed to form a firm clot after which the coverslip was placed in a chamber as follows: The 45 mm x 50 mm coverslip was covered with a rubber gasket and then another coverslip. This was removed from the petri dish and placed between the metal plates of the Rose chamber, and then these metal plates were screwed together. The coverslip of 25 mm diameter was removed from the petri dish with a blunt forceps and spatula and placed in the top of the Sykes-Moore chamber which was in another petri dish. Then the silicone rubber gasket was added, then another coverslip, and then the bottom part of the chamber. This ensemble was then removed from the petri dish and screwed together with the wrench assembly. A drop of Hanks BSS (pH 7.2-7.6) was injected into each of the chambers, and they were incubated at 37°C. overnight. All procedures stated above were performed in the tissue culture room under sterile conditions until the flasks were prepared. When the tissue was placed in the chambers, sterile procedures were also used until the second coverslip was added in the case of the Rose chamber or until the bottom part of the chamber was in place in the case of the Sykes-Moore chamber.

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g. Dessicated chick plasma (Difco Laboratories, Detroit, Michigan)

h. Flow Laboratories, Rockville, Maryland.

The next day each chamber was inspected for "turners" under 100x magnification, and if present, the chamber was filled with Hanks BSS (pH 7.4) and placed under the microscope at room temperature and with the microscope light turned on. The chamber was allowed to stay in this position for at least 10 minutes. A baseline time was then determined for each of 1-3 "turners" in the chamber. This was done by recording the time it took for the "turner" to complete 10 rotations. This measurement was recorded every 5 minutes for 30 minutes. If these values did not vary by more than 10%, they were averaged and this average was the baseline time. Approximately 2 ml of the test solution (Hanks BSS of a specified pH) was then injected into the chamber, completely replacing the Hanks solution of pH 7.4, and the same measurements were recorded over another 30 minute period (Sometimes the "turners" were observed for longer than 30 minutes). After the latter values were tabulated, they were averaged over 15 minute intervals and the averages were divided by the baseline time to obtain the percent of the baseline time. Graphs were then plotted showing the relationship between percent of the baseline time and time, or between percent of the baseline time and pH. It is to be noted that an increase in percent of the baseline time represents an increase in time required for 10 rotations and thus a decrease in speed of the "turner". On the other hand, a decrease in percent of the baseline time represents a decrease in time required for 10 rotations and thus an increase in speed of the "turner".

RESULTS

Although several different methods were used to obtain tissue for cultivation, including the use of animal tissue, bronchial scraping from excised human lung tissue, and tracheal scrapings obtained at bronchoscopy, nasal polyps and accessory sinus mucous membrane seemed to have the most "turners". Six groups of nasal polyps and one group of maxillary sinus epithelium were obtained. Two of the groups of nasal polyps were cultured but failed to survive overnight in each case. Of interest, however, is that one of these groups of polyps was taken from a patient who was allergic to penicillin and then placed in a medium containing penicillin. The other groups of polyps and maxillary sinus tissue produced numerous "turners", the number of "turners" in each flask varying from none to over 100, but, due to technical difficulties and to the use of a few "turners" in another study, only two of the groups were used in these experiments.

The two groups of polyps were obtained from the following patients:

Patient S.S.: 50 yr. old white male with chronic sinusitis associated with allergy. The polyps were obtained from the ethmoid sinus.

Eighteen Falcon flasks and 22 chambers were prepared from the tissue, and more than 64 "turners" were found (only four of them were found in the chambers). Of these only six were finally used for this study (Turners No. 1,2,3,4,5,6).

Patient J.F.: 34 yr. old white female with hyperplastic nasal polyps without an allergic history.

Fifteen Falcon flasks were prepared from the tissue and over 200 "turners" were found. However, only thirteen "turners" were utilized in this study.
(Turners No. 7,8,9,10,11,12,13,14,15,16,17,18,19).

In my study of the effect of pH on ciliary activity, I perfused 8 "turners" with Hanks BSS solutionsof pH less than 7.4 after having obtained a baseline time for 10 rotations for each one of them in a solution of pH 7.4 (one baseline time was obtained at pH 7.3). Seven of these "turners" were perfused with Hanks solution of pH 6.5 and one "turner" was perfused with a solution of pH 6.8. The results of these studies are shown in Tables II-V. The calculations of the percent of baseline time for each "turner" at each 15 minute time interval as described on page 16 are shown in Tables VI-XIII.

I then studied, in a similar way, the effect of solutions of pH greater than 7.4 on 9 "turners". The baseline times in 6 "turners" were determined at pH 7.4, but in three of the "turners" the baseline times were determined at pH 7.2 or pH 7.3. Three "turners" were perfused with Hanks solution of pH 7.8, three were perfused with Hanks solution of pH 8.2, and three were perfused with Hanks solution of pH 8.5. The results are shown in Tables XIV-XVII and the calculations of percent of baseline time are shown in Tables XVIII-XXVI.

Since some of my baseline studies had been carried out using a solution of pH 7.2 or 7.3 rather than pH 7.4, I perfused three "turners" with a Hanks BSS solution of pH 7.4 after having obtained a baseline time at pH 7.2 to see if there was any effect on ciliary activity of solutions within the pH range 7.2-7.4. The results are shown in Table XXVII and the calculations of percent of baseline time are shown in Tables XXVIII-XXX.

A graph (Figure 7) was then drawn showing, for each "turner", the relationship between the percent of baseline time for 10 rotations and the time after addition of the test solution of specified pH. Then the percents of baseline times for all of the several "turners" at each pH at each 15 minute interval were averaged and a second graph (Table XXXI and Figure 8) was drawn showing the relationship between these averages and the time after addition of the test solution. A third graph was drawn showing how the percent of baseline time varies with the pH of the test solution at time after addition of the test solution= 30 minutes. (Table XXXII, Figure 9).

As can be seen from the above-mentioned tables and graphs, there is an increase in percent of baseline time with a decrease in pH of the solution from pH 7.4 and a decrease in percent of baseline time with an increase in pH of the solution above pH 7.4. This implies a decrease in speed of rotation of the "turners"

or a decrease in ciliary activity with a decrease in pH below 7.4 and an increase in speed of rotation or an increase in ciliary activity with an increase in pH above 7.4. This may also be stated as a gradual increase in ciliary activity as the pH of the solution increases from 6.5 to 8.5.

STATISTICAL EVALUATION

In the statistical analysis I calculated means and variances for each set of observations within each experiment. Since the levels of the averages differed from experiment to experiment, it appeared that the proper basis for comparisons was the differences in the averages, by experiment. I was also interested in studying the variability in the time required for 10 rotations, by experiment, and across experiments. Although the "turners" were selected for their low variability in rotational time during the baseline perfusion, it is of interest that, by experiment, the variabilities of the times of rotation in the baseline samples were usually similar to those in the test samples. There were notable exceptions, however.

In order to determine if there was any tendency for the averages to increase or decrease from a change of solution alone without significantly changing the pH and also to determine if a small pH change would affect the rotation, experiments 12, 13, and 14 were run. Perhaps more should have been run. The averages in experiments 12, 13, and 14 show no consistent difference (i.e. a t-test of the differences between the means (equivalent to a test of the difference between the overall mean in the three experiments after change of solution and the overall mean in the baseline periods of the three experiments) gave a value of -.351 for 36 degrees of freedom which is not statistically significant.

While an overall t-test of the average difference was used in evaluating experiments 12,13, and 14, this approach did not appear to be suitable for examining the differences in the other experiments. Instead, in each set of experiments(i.e. pH 7.4 to a lower pH; pH 7.4 to a higher pH) the difference between averages (baseline minus test averages) was calculated for each experiment and a t-test was used with these differences. This was an approximate procedure in view of the fact that the number of observations varied, both within and between experiments, and in some cases the variances of the observations within an experiment were quite different. The results were:

a. pH 7.4 to a lower pH

t (7 degrees of freedom) = -2.01 $.05 < P < .10$

b. pH 7.4 to a higher pH

t (8 degrees of freedom) $P < .01$

These t values are both significant and indicate that there is a significant increase in rotational time in a. and a significant decrease in rotational time in b. (Table XXXIII)

DISCUSSION

There are several methods for studying ciliary activity in mammals. One of the oldest methods is that of indirectly measuring ciliary activity by measuring mucous transport rate. This can be done by following the movement of particles in the mucus, either natural ones such as cells or bubbles, or artificial ones such as dye particles, soot, radioactive particles or radio-opaque particles. This is done either on freshly excised respiratory epithelium or in the living animal, and the rate of movement of the particle is observed either by light microscopy, X-ray photography or tracer methods depending on the type of particle used. The problems involved with this technique are related to the influence of the size, shape, weight and chemical nature of the particle as well as to the rate of transport of the particle which varies widely in different areas of the epithelium.[44] If particles are insufflated then a very moderate amount should be used as the normal cleansing mechanism can be blocked by excessive amounts (e.g. greater than 1.5 mg/lung in the rat).[44] Another source of error in this method, when it is being used to measure ciliary activity, is based on the fact that mucous flow may not always be directly proportional to the frequency of ciliary beat.[10]

Ciliary motility may also be measured more directly by observing the flickering light reflex from the cilia and measuring this by direct visualization, stroboscopy, cinephotography or through the use of photoelectrical equipment.[17,42,44] Direct visualization can pick up only large changes in beat frequency because the normal beat frequency is so high. Stroboscopy is also difficult to use because there is a significant variability in the flicker frequencies of adjacent areas in an intact membrane.[44] In addition, if the tissue is in culture, the border is often 15-20 cilia deep and it is difficult to "stop" this layer when each cilium is in a different part of the cycle.[3] Also, certain of the beat frequencies are difficult for the human eye to adjust to when using the stroboscope.[44] Cinephotography is probably the best method for measuring ciliary activity.

The study of human ciliated epithelium is most easily undertaken through the use of tissue culture, and the activity of ciliated cells in culture can be studied using any of the above methods(except the mucous flow rate). It can also be studied by still another method. This is the rotating explant method (REM), fully described by Ballenger in 1963, upon which I based my techniques for cultivating ciliated cells and studying ciliary activity. Although his basic idea seems to be valid--that these rotating cells are performing work and that, should this rotation stop, it would be a reflection of either a decrease

in frequency, amplitude, or co-ordination of the beat-- there are many problems inherent in the technique. One of the major difficulties is in obtaining tissue for the use of this method. Although I have been able to obtain "turners" from animal material (monkey, cat, and rabbit tracheae) the yield is extremely low. Therefore the use of human tissue, which produces numerous "turners", has proved more practical. Dr. Ballenger uses tracheal scrapings which he obtains from children at operation. I have also attempted to obtain tracheal scrapings at the time of tonsillectomy and bronchoscopy but this is not a technique accomplished easily by other surgeons since just the right depth and length of scraping must be obtained. In addition, before bronchoscopy topical anesthetics are sprayed into the trachea,[i.] and during bronchoscopy the epithelium is traumatized by the passage of the bronchoscope. Other methods of obtaining human tissue such as scraping the nasal mucosa and scraping the bronchi of excised lung tissue are also unsatisfactory, the former because it is difficult to do without topical anesthesia and the latter because the lung is usually not removed unless cancerous or tuberculous and these types of tissue are undesirable. Nasal polyps, when removed, are relatively easy to acquire although they must then be washed in Hanks solution containing higher concentrations of antibiotics than usual because of the fact that they are highly contaminated. The only problem

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[i.] Corssen and Allen have shown that local anesthetics may stop ciliary activity depending on the anesthetic used and the concentration of it. (7,9)

encountered in using this type of tissue is that the majority of each polyp is connective tissue thus providing only a small percentage of epithelial cells for a given amount of minced tissue. Because Ballenger had predominately epithelial cells in his scrapings he could place this tissue directly into plasma clots in hanging-drop slides and have a majority of his clots have "turners" in them. Becasue of this preponderance of connective tissue in the polyps, in my study, when using nasal polyps, much larger cultivation containers were needed to obtain a reasonable number of "turners". For example when I put explants from patient S.S. in 22 chambers (bypassing the Maximow slides) only four "turners" were found. I therefore found it practicable to culture the explants in Falcon flasks and the tissue seemed to thrive well in the medium used.

Difficulties also present themselves in getting the "turners" out of the flasks since both the flask and the explant must remain sterile. Perhaps a suitable culture container that was made out of plastic similar to that of the Falcon flask but which had a wider opening with a screw cap (so that there was no evaporation) could facilitate this transfer.

Still another problem arises with the use of the plasma clot. It is one of the older methods of tissue culture and has several inherent disadvantages. In my study, I noticed that the "turners" could not be perfused the same day that they were transferred from the flask to the chamber because the clots would come off the coverslip when the Hanks BSS was added. I also observed that

the chick plasma-chick embryo extract clot did not maintain cell viability as well as did the medium 199-fetal bovine serum mixture used in the flasks--even overnight. The thickness of the plasma clot also hinders the use of the phase microscope in observing the cells. Despite these hindrances, the clot still provides the only method reported to date of anchoring the "turners" to the coverslip. Perforated cellophane has been found to impede the rotation of the "turners"[j.] but perhaps the use of nylon mesh for this purpose could be investigated.

A fourth problem is related to the fact that a few "turners" did not seem to be rotating at a constant speed when observed in the initial Hanks BSS, a fact which Ballenger does not report in any of his articles.

Although I tried to follow Ballenger's technique as closely as possible after the "turners" were placed in the chamber, I did add the test fluid by injecting approximately 2 ml of the fluid with a syringe over about a one minute period rather than perfusing 4 ml of the test fluid over a 10 minute period as Ballenger does. It is possible, of course, that the more rapid infusion of the test solution could have affected the rotation of the "turners". This is unlikely in view of the immediate and persistent change in rotation of the "turners" seen after the addition of the fluid in my experiments.

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j. Personal communication from Dr. J.J. Ballenger.

In general, then, I found the KEM technique a difficult one to use for the study of ciliary activity. Perhaps a better method of studying ciliary activity in tissue culture would be to grow the ciliated epithelium on coverslips in chambers using medium 199-fetal bovine serum as the nutrient medium and then to determine the beat frequency of the cilia using cinephotography.

From the results of my experiments I can conclude that an increase in pH or a decrease in hydrogen ion concentration of a solution from pH 7.4 causes an increase in ciliary activity and that a decrease in pH or an increase in hydrogen ion concentration of a solution from pH 7.4 causes a retardation of ciliary activity. It is difficult to compare these results with others obtained for mammalian cilia. As can be seen from the summary of reports on the effect of pH on mammalian ciliary activity given on pages 11-12 and in Table I, some authors give only an optimal pH while others consider cessation of ciliary activity as the end point for the effect of a solution of a certain pH. When actual pH values are given, most authors reported 6.0-6.5 as the pH of a solution below which there was slowing of ciliary activity. My results are compatible with this in that I found a gradual slowing of ciliary activity from pH 7.4 down to pH 6.5. Only Hilding and Negus, however, report an increase in ciliary activity with solutions with pH's in the range of 8.5-9.0. Other authors such as Kent found a slowing of ciliary activity with

solutions of pH greater than 8, and Spock found a cessation of ciliary activity with solutions of pH greater than 9.0. In view of this discrepancy it is necessary to study the effect of alkaline pH solutions on more "turners" to be conclusive. However, my data seem to agree with those of Negus and Hilding. It is entirely possible, though, that this increase in ciliary activity may be only a prelude to cell death and a decrease in ciliary activity as has been found with other agents upon prolongation of the time the cilia are in contact with the solution.[7,8] Should this increase in ciliary activity with solutions of pH greater than physiologic pH be further substantiated it would be advisable from this standpoint for all topical preparations used in the respiratory tract to be of pH greater than 7.4.

The importance of ciliary activity for the cleansing mechanism of the respiratory tract cannot be underestimated. The mucous transport of the respiratory tract depends on ciliary motility and the physical properties of the mucus, and mucous transport and phagocytosis are the only means of keeping the respiratory tract clean. Without mucous transport, stagnation of secretions occurs, thus predisposing to infections. Often pulmonary atelectasis, post-operative pneumonia as well as sinusitis and other upper respiratory tract infections have been attributed in many cases to failure of ciliary currents.[36] Perhaps still other disease such as cystic fibrosis may be due in part to a failure of ciliary activity.

SUMMARY

Ciliated epithelium obtained from human nasal polyps was placed in tissue culture after which the effect of pH on the ciliary activity was studied utilizing the rotating explant method of Ballenger. A reduction in pH of the Hanks Balanced Salt Solution from pH 7.4 down to 6.8 or less resulted in a slowing of ciliary activity, and an increase in pH of the Hanks Balanced Salt Solution from pH 7.4 to 7.8 or greater resulted in an increase in ciliary activity during the first thirty minutes after the cilia were placed in contact with the test solutions.

TABLES

TABLE I

Date	Investigator	Animal used	Ciliary activity		
			pH < 7.4 (pH 7.4 used as baseline)	pH > 7.4	Optimum pH
1866	Kuhne	?	acid stopped ciliary activity		
1930	Chase & Glaser	*Paramecium*			5.1-6.3
1961	Dryl	*Paramecium*			5.1-6.3
1928	Gray	*Mytilus*	decreased with decrease in pH to 5.6	increased with increase in pH to 9.0	
1934	Tomita	*Ostrea*	fall in activity at 5.5		
1965	Das	Frog (pharynx)	decrease to 6.5 increased cil. motil. below 6.0 decreased C.A.	decreased	
1934	Negus	Rabbit (resp. ep.)	stopped at pH 6.4	cilia beating at 8.5	
1963	Hilding	?	decreased at pH 6.0	increased at 9.0	
1965	Kent	Human, rabbit guinea pig (resp. ep.)	pH 6.0 decreased activity	pH above 8.0 decreased activity	
1966	Kensler & Battista	rabbit trachea			6.5-8.5
1966	Spock	Rabbit trachea	stopped pH 1.0	stopped pH 9.0	

TABLE II

Turners No. 7,8,9
(They were counted in the same chamber)

Time(min) after add Hanks BSS	Time for 10 rotations (sec)			Remarks
	7	8	9	
0				pH 7.4 Hanks BSS added
70	36.4	27.4	31.2	
75	38.0	28.6	31.4	
80	39.0	28.8	31.2	
85	40.2	29.3	31.3	
90	41.6	29.9	31.3	
95	42.2	29.9	30.6	
100	40.4	30.0	29.8	
105	41.0	30.3	30.0	
0	50.4	30.9	33.2	pH 6.5 Hanks BSS added
5	52.8	32.2	33.6	
11	54.2	31.8	34.2	There is a possibility that No. 7 was moving around during this time
16	52.5	31.8	33.8	
20	51.4	31.7	33.4	
25	52.7	31.5	33.5	
30	54.3	31.4	33.2	
35	54.8	31.6	33.6	

TABLE III

Turners No. 10,11
(They were counted in the same chamber)

Time(min) after add Hanks BSS	Time for 10 rotations (sec)		Remarks
	10	11	
0			pH 7.4 Hanks BSS added
45	33.8	30.0	
50	33.5	29.6	
55	33.4	29.4	
60	33.8	28.9	
65	34.3	28.9	
70	34.0	28.6	
75	34.4	28.9	
0	34.4	30.1	pH 6.5 Hanks BSS added
5	33.2	30.3	
10	33.4	30.6	
15	31.8	31.0	
20	32.8	31.2	
25	33.3	30.8	
30	32.6	31.4	

TABLE IV

Turners No. 15,16
(They were counted in the same chamber)

Time(min) after add Hanks BSS	Time for 10 rotations (sec)		Remarks
	15	16	
0			pH 7.4 Hanks BSS added
46	35.6	38.6	
50	35.5	39.0	
55	36.2	39.2	
62	37.8	40.0	
67	38.2	34.6	
70	39.0	32.2	
77	42.2	38.6	
0			pH 6.5 Hanks BSS added
3	37.4	37.2	
8	39.8	37.2	
13	41.8	38.4	
19	42.6	38.8	
26	45.2	39.2	
30	45.8	39.2	
33	46.6	39.6	No. 15 beginning to show signs of cell death.

TABLE V

Turner No. 1

Time(min) after add Hanks BSS	Time for 10 rot. (sec)	Remarks
0		pH 7.3 Hanks BSS added *
4	32.6	
5	31.4	
10	24.7	
15	23.8	
20	22.8	
25	22.8	
30	23.6	
35	24.3	Average=24.1=Baseline time
40	22.6	
46	25.0	
50	24.7	
55	25.2	
60	24.9	
65	24.6	
73	24.0	
76	24.0	
0	26.0	pH 6.8 added**
1	25.3	
3	24.2	
6	23.6	
10	21.4	
15	23.8	
20	24.4	
26	24.8	
30	25.0	
36	25.0	
46	25.0	
56	25.0	

* The first 10 minutes were eliminated from the calculations since this time appears necessary for the tissue to become acclimated to its environment.

** This pH was 6.8 or less. The pH meter was not properly adjusted at the time of this reading but according to the phenol red indicator which was present in the solution the pH was 6.8 or less.

TABLE VI
(Turner No. 7), pH 7.4-6.5

Time interval	Av. time (sec)	% baseline time
Baseline time= 39.8 sec.		
0-15	52.5	131.9
15-30	52.7	132.4
30-35	54.8	137.7

TABLE VII
(Turner No. 8), pH 7.4-6.5

Time interval	Av. time (sec)	% baseline time
Baseline time= 29.3 sec.		
0-15	31.6	107.8
15-30	31.6	107.8
30-35	31.6	107.8

TABLE VIII
(Turner No. 9), pH 7.4-6.5

Time interval	Av. time (sec)	% baseline time
Baseline time= 30.8 sec.		
0-15	33.7	109.4
15-30	33.5	108.8
30-35	33.6	109.1

TABLE IX
(Turner No. 10), pH 7.4-6.5

Time interval	Av. time (sec)	% baseline time
Baseline time= 33.9 sec		
0-15	33.2	97.9
15-30	32.9	97.0

TABLE X
(Turner No. 11), pH 7.4-6.5

Time interval	Av. time (sec)	% baseline time
Baseline time= 29.2 sec.		
0-15	30.5	104.4
15-30	31.1	106.5

TABLE XI
(Turner No. 15), pH 7.4-6.5

Time interval	Av. time (sec)	% baseline time
Baseline time= 37.8 sec.		
0-15	39.7	105.0
15-30	44.5	117.7
30-35	46.6	123.3

TABLE XII
(Turner No. 16), pH 7.4-6.5

Time interval	Av. time (sec)	% baseline time
Baseline time= 37.4sec.		
0-15	37.9	101.3
15-30	39.1	104.5
30-35	39.6	105.9

TABLE XIII
(Turner No. 1), pH 7.3-6.8

Time interval	Av. time (sec)	% baseline time
Baseline time= 24.1 sec.		
0-15	24.0	99.6
15-30	24.7	102.5
30-45	25.0	103.7
45-55	25.0	103.7

TABLE XIV

Turners No. 4,5,6
(They were counted in the same chamber)

Time(min) after add Hanks BSS	Time for 10 rotations (sec)			Remarks
	4	5	6	
0				pH 7.4 Hanks BSS added
3	24.6	40.4	44.0	* First 10 min omitted from calculations (see note on Table V)
10	24.4	39.0	38.8	
20	24.4	38.2	40.8	
25	24.2	37.7	41.6	
31	24.0	37.2	40.4	
39	24.2			
40	24.0	32.8	40.2	
45	24.0	32.7	39.8	
62	24.0	34.0	39.8	
0				pH 7.8 Hanks BSS
1	24.7	35.0	36.4	
5	21.9	34.4	34.6	
10	20.8	34.6	34.8	
15	21.0	34.6	35.3	
21	21.2	35.3	35.4	
25	21.4	29.9	35.8	
30	21.5	31.4	36.6	
36	21.7	31.7	36.2	

TABLE XV

Turners No. 17,18,19
(They were counted in the same chamber)

Time (min) after add Hanks BSS	Time for 10 rotations (sec)			Remarks
	17	18	19	
0				pH 7.4 Hanks BSS added
50	36.8	33.8	31.3	
55	32.4	34.4	32.0	
60	35.4	35.4	32.8	
66	37.2	35.8	33.0	
72	38.0	35.6	34.3	
75	38.6	35.6	34.0	
80	38.8	35.2	34.2	
0				pH 8.2 Hanks BSS added
2	35.6	30.8	28.4	
5	31.4	29.4	26.7	
11	28.7	29.4	25.9	
15	29.4	30.3	26.4	
20	29.6	29.7	27.6	
25	30.4	29.9	28.4	
30	30.8	30.1	29.6	
35	31.2	30.2	30.4	
42	31.2	30.3	29.8	
45	31.6	30.2	27.6	
52	31.7	30.8	28.3	
55	31.8	30.7	28.8	
120	30.9	32.6	31.0	Chamber was sitting off the microscope

TABLE XVI

Turner No. 1
(second perfusion)

Time(min) after add Hanks BSS	Time for 10 rotations (sec)	Remarks
0		pH 7.3 Hanks BSS added
3	25.0	
14	25.9	
18	27.0	Average=28.2 sec
23	27.9	= Baseline time
33	29.8	
38	29.6	These values
43	30.3	appear to be
48	29.8	increasing slightly
0		pH 8.5 Hanks BSS added *
1	27.8	
6	25.7	
11	25.0	
16	25.0	
21	23.7	
26	22.2	
46	18.8	
51	17.4	
61	19.2	
66	19.2	
71	19.6	
76	19.8	
81	20.0	
86	20.8	
91	21.0	

* The pH meter was not adjusted properly when this pH was read. According to the phenol red indicator in the solution the pH was 8.0 or greater and probably the pH was approximately 8.5.

TABLE XVII

Turners No 2,3
(They were counted in the same chamber)

Time(min) after add Hanks BSS	Time for 10 rotations (sec)		Remarks
	2	3	
0			pH 7.2 Hanks added
2	48.5	39.4	*
10	53.3	36.8	
15	50.4	39.6	
20	46.2	40.4	
26	43.4	39.8	
30	42.4	40.8	
0			pH 8.5 Hanks added
4	38.6	40.0	
6	36.4	40.1	
10	34.2	38.6	
15	32.6	38.0	
20	30.9	33.4	
26	33.0	32.0	
30	31.5	31.6	
37	33.4	31.2	
40	32.4	31.7	
44			pH 7.2 Hanks added
48	32.6	32.6	
50	32.6	33.8	
55	33.2	36.0	
60	33.6	38.0	
68	33.3	39.8	
70	34.0	37.8	
75	35.2	41.2	
80		42.4	
84	36.6		

* First 10 min omitted from calculations (see note on Table V)

TABLE XVIII
(Turner No. 4), pH 7.4-7.8

Time interval	Av. time (sec)	% baseline time
Baseline time= 24.2 sec.		
0-15	22.1	91.3
15-30	21.4	88.4
30-40	21.7	89.7

TABLE XIX
(Turner No. 5), pH 7.4-7.8

Time interval	Av. time (sec)	% baseline time
Baseline time= 35.9 sec.		
0-15	34.6	96.4
15-30	32.2	89.7
30-40	31.7	88.3

TABLE XX
(Turner No. 6), pH 7.4-7.8

Time interval	Av. time (sec)	% baseline time
Baseline time= 40.2 sec.		
0-15	35.3	87.8
15-30	35.9	89.3
30-40	36.2	90.0

TABLE XXI
(Turner No. 17), pH 7.4-8.2

Time interval	Av. time (sec)	% baseline time
Baseline time= 36.7 sec.		
0-15	31.3	85.3
15-30	30.3	82.6
30-45	31.3	85.3
45-55	31.8	86.6
120	30.9	84.2

TABLE XXII
(Turner No. 18), pH 7.4-8.2

Time interval	Av. time (sec)	% baseline time
Baseline time= 35.0		
0-15	30.0	85.7
15-30	29.9	85.4
30-45	30.2	86.3
45-55	30.8	88.0
120	32.6	93.1

TABLE XXIII
(Turner No. 19), pH 7.4-8.2

Time interval	Av. time (sec)	% baseline time
Baseline time= 33.1		
0-15	26.8	81.0
15-30	28.5	86.1
30-45	29.3	88.5
45-55	28.6	86.4
120	31.0	93.6

TABLE XXIV
(Turner No. 1), pH 7.3-8.5

Time interval	Av. time (sec)	% baseline time
Baseline time= 28.2 sec.		
0-15	26.2	92.9
15-45	23.6	83.7
45-60	18.1	64.2
60-75	19.3	68.4
75-90	20.2	71.6
91	21.0	74.5

TABLE XXV
(Turner No. 2), pH 7.2-8.5
then back to 7.2

Time interval	Av. time (sec)	% baseline time
Baseline time= 47.1 sec.		
0-15	35.4	75.2
15-30	31.8	67.5
30-40	32.9	69.8
44	Hanks pH 7.2	
45-60	33.0	70.1
60-75	34.2	72.6
75-85	36.6	77.7

TABLE XXVI
(Turner No. 3), pH 7.2-8.5
then back to 7.2

Time interval	Av. time (sec)	% baseline time
Baseline time= 39.5 sec.		
0-15	39.2	99.2
15-30	32.3	81.8
30-40	31.4	79.5
44	Hanks pH 7.2	
45-60	35.1	88.9
60-75	40.3	102.0
75-85	42.4	107.3

TABLE XXVII

Turners No. 12,13,14
(They were counted in the same chamber)

Time(min) after add Hanks BSS	Time for 10 rotations (sec)			Remarks
	12	13	14	
0				pH 7.2 Hanks added
65	52.4	40.2	34.6	
70	49.2	41.4	35.2	
76	47.8	42.4	35.6	
81	48.8	43.2	32.8	
86	49.4	44.2	33.3	
90	49.0	38.8	33.5	
95	49.5	38.8	34.0	
0				pH 7.4 Hanks added
2	57.2	34.8	35.6	
5	56.5	34.0	35.5	
11	54.4	33.6	35.6	
17	53.6	33.4	36.2	
21	52.8	33.6	35.6	
25	52.4	34.8	33.7	
30	52.4	34.8	33.7	Chamber removed from microscope during this interval.
85	58.6	36.4	28.5	

TABLE XXVIII
(Turner No. 12), pH 7.2-7.4

Time interval	Av. time (sec)	% baseline time
Baseline time=49.4 sec.		
0-15	56.0	113.4
15-30	52.8	106.9
85	58.6	118.6

TABLE XXIX
(Turner No. 13), pH 7.2-7.4

Time interval	Av. time (sec)	% baseline time
Baseline time= 41.3 sec.		
0-15	34.1	82.6
15-30	33.9	82.1
85	36.4	88.1

TABLE XXX

(Turner No. 14), pH 7.2-7.4

Time interval	Av. time (sec)	% baseline time
Baseline time= 34.1		
0-15	35.6	104.4
15-30	34.8	102.0
85	28.5	83.6

TABLE XXXI

pH	Time interval	Percents of baseline times	Av. % of Baseline time S.D.
6.5	0-15	101.3,131.9,107.8,109.4,97.9,104.4,105.0	108.2 ± 11.1
	15-30	104.5,132.4,107.8,108.8,97.0,106.5,117.7	110.7 ± 11.4
	30-45	105.9,137.7,107.8,109.1,123.3	116.8 ± 13.6
6.8	0-15	99.6	99.6
	15-30	102.5	102.5
	30-45	103.7	103.7
	45-60	103.7	103.7
7.4	0-15	113.4,82.6,104.4	100.1 ± 15.8
	15-30	106.9,82.1,102.0	97.0 ± 13.1
	90	118.6,88.1,83.6	96.8 ± 19.0
7.8	0-15	91.3,96.4,87,8	91.8 ± 4.3
	15-30	88.4,89.7,89.3	89.1 ± 0.7
	30-45	89.7,88,3,90.0	89.3 ± 0.9
8.2	0-15	85.3,85.7,81.0	84.0 ± 2.6
	15-30	82.6,85.4,86.1	84.7 ± 1.8
	30-45	85.3,86.3,88.5	86.7 ± 1.6
	45-60	86.6,88.0,86.4	87.0 ± 0.9
	120	84.2,93.1,93.6	90.3 ± 5.3
8.5	0-15	92.9,75.2,99.2	89.1 ± 12.4
	15-30	83.7,67.5,81.8	77.7 ± 8.8
	30-45	69.8,79.5	74.6 ± 6.8

TABLE XXXII

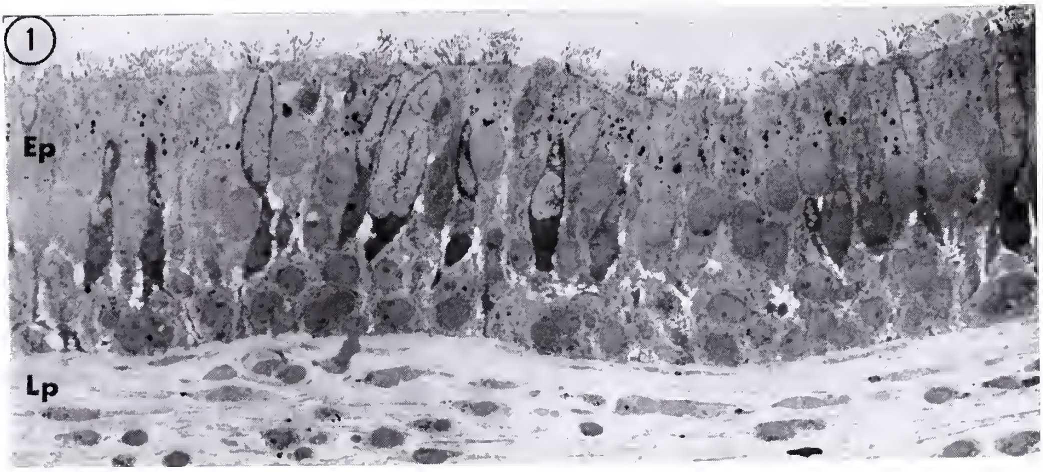
pH	6.5	6.8	7.4	7.8	8.2	8.5
% of baseline time	136.4 107.2 107.8 96.2 107.5 121.2 104.8	103.7	106.1 84.3 98.8	88.8 87.5 91.0	83.9 86.0 89.4	78.7 66.9 80.0

TABLE XXXIII

BASELINE					TEST SOLUTION(first 30 min)			
pH	Patient & Turner No.	No.obs. (n)	Av. Time	Sample variance	pH	No.obs.	Av. time	Sample variance
7.4	J.F.-7	8	39.8	3.77	6.5	7	52.6	1.96
7.4	J.F.-8	8	29.3	0.930	6.5	7	31.6	0.165
7.4	J.F.-9	8	30.8	0.406	6.5	7	33.6	0.126
7.4	J.F.-10	7	33.9	0.142	6.5	7	33.1	0.642
7.4	J.F.-11	7	29.2	0.245	6.5	7	30.8	0.222
7.4	J.F.-15	7	37.8	5.61	6.5	6	42.1	10.2
7.4	J.F.-16	7	37.4	8.38	6.5	6	38.3	0.850
7.3	S.S.-1	14	24.1	0.742	6.8	9	24.3	1.72
7.2	J.F.-12	7	49.4	2.02	7.4	7	54.2	3.85
7.2	J.F.-13	7	41.3	4.49	7.4	7	34.0	0.728
7.2	J.F.-14	7	34.1	1.07	7.4	7	35.1	1.05
7.4	S.S.-4	8	24.2	0.031	7.8	7	21.8	1.78
7.4	S.S.-5	7	35.9	7.21	7.8	7	33.6	4.46
7.4	S.S.-6	7	40.2	0.773	7.8	7	35.6	0.573
7.4	J.F.-17	7	36.7	5.02	8.2	7	30.8	5.23
7.4	J.F.-18	7	35.1	0.545	8.2	7	29.9	0.256
7.4	J.F.-19	7	33.1	1.33	8.2	7	27.6	1.74
7.3	S.S.-1	8	28.2	4.08	8.5	6	24.9	3.56
7.2	S.S.-2	5	47.1	21.5	8.5	7	33.9	7.61
7.2	S.S.-3	5	39.5	2.47	8.5	7	36.2	14.2

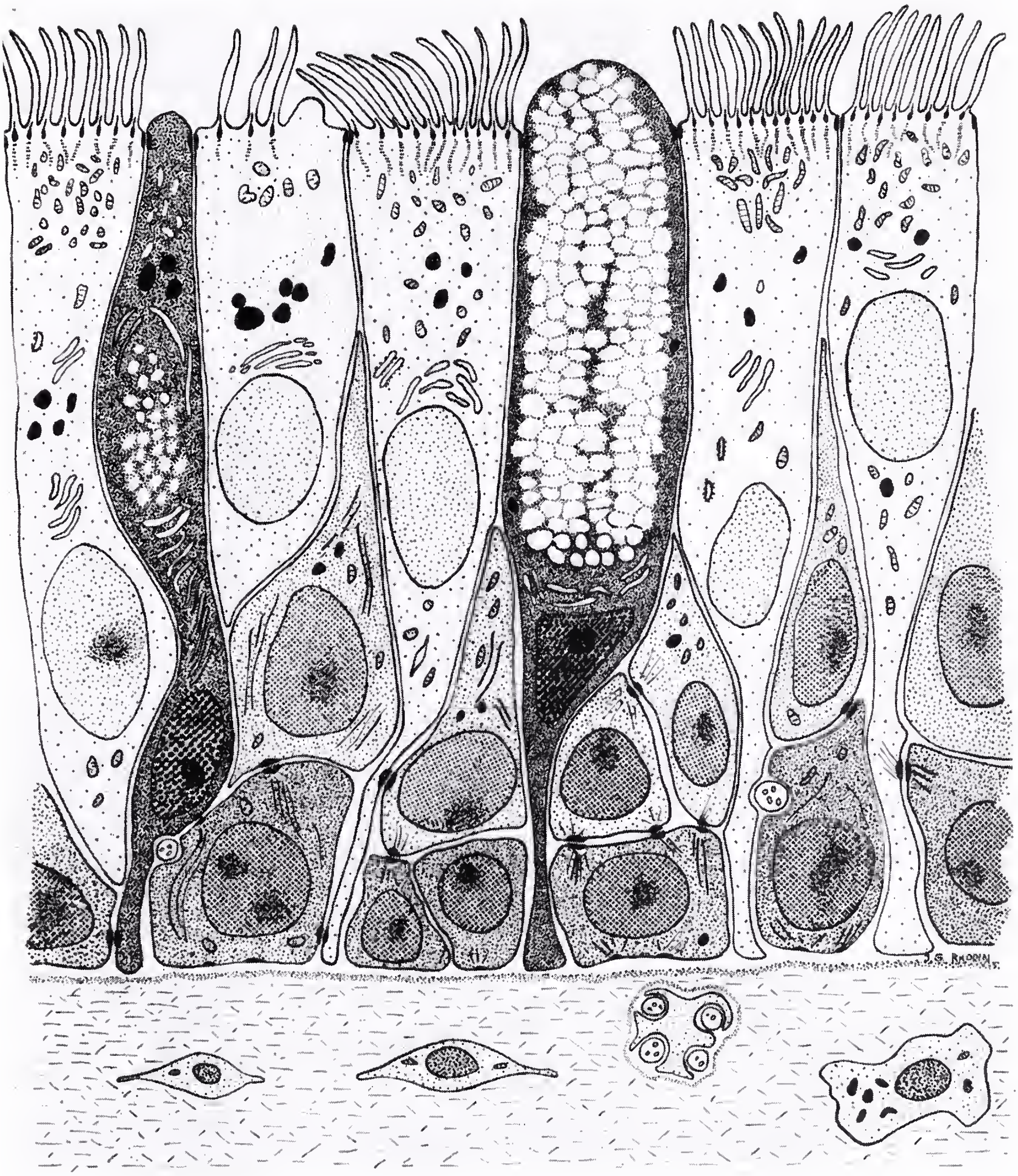
ILLUSTRATIONS

Figure 1



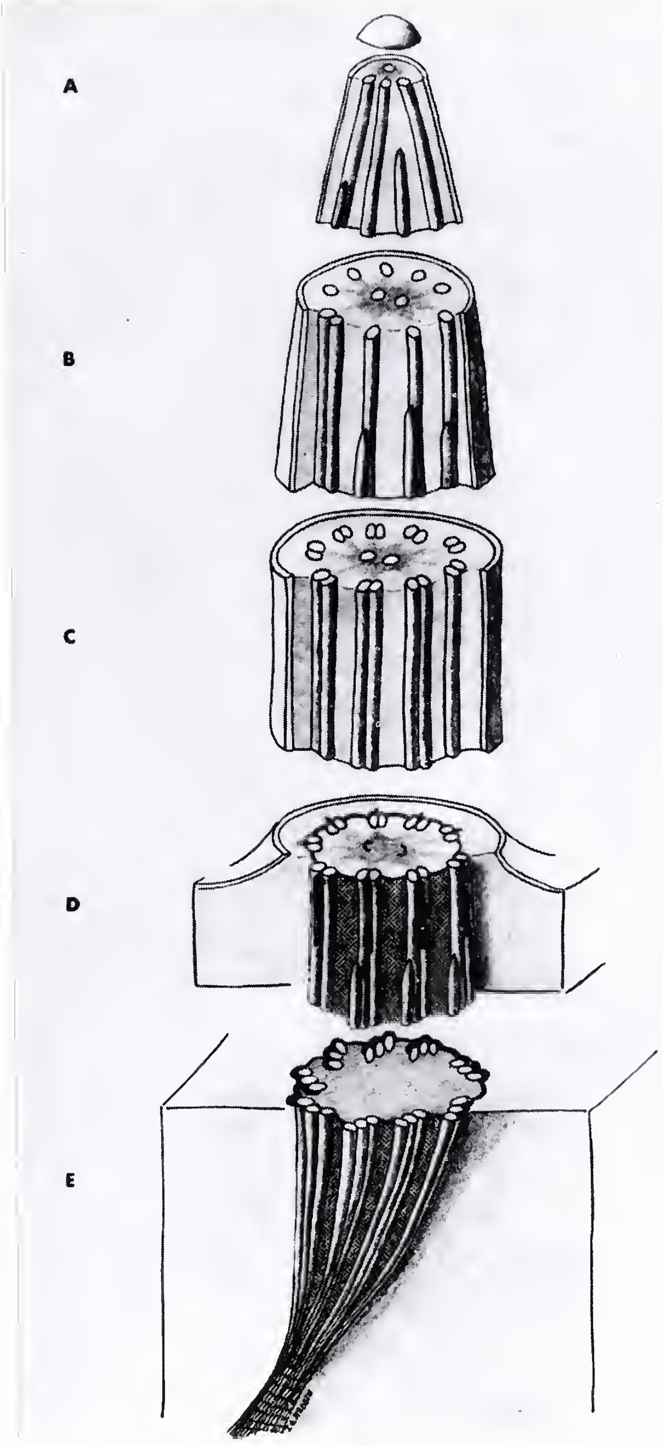
Longitudinal section of human tracheal epithelium (Ep) with part of the lamina propria (Lp). Electron micrograph. (Magnification, x650) (Reproduced from *American Review of Respiratory Diseases*, v. 93, no. 3, part 2, page 2)

Figure 2



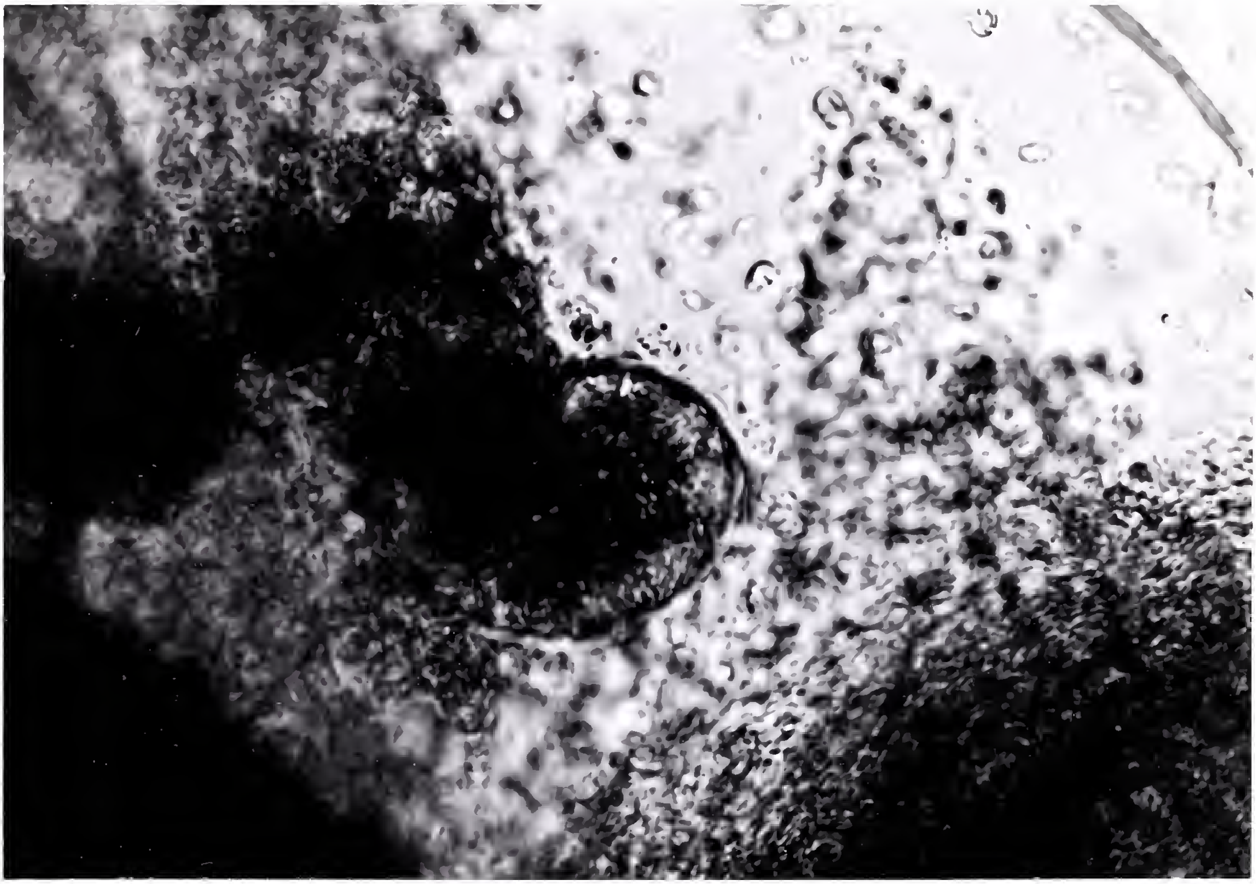
Schematic representation of the pseudostratified ciliated epithelium of the human trachea. The drawing is based on a slightly simplified tracing of an electron micrograph. The four cell types may be seen.
(Reproduced from American Review of Respiratory Diseases v. 93 no. 3, part 2, page 13.)

Figure 3



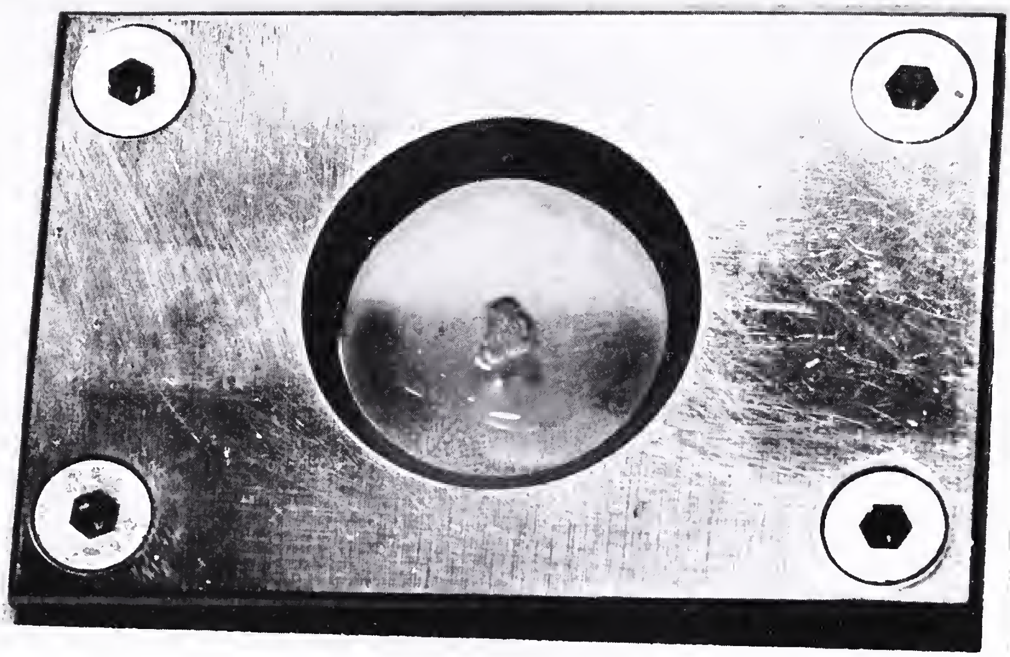
This drawing summarizes the fine structure of a human tracheal cilium and its basal body. It represents Rhodin's interpretation of the three-dimensional orientation of mainly the peripheral filaments, in the tip of the cilium (A and B), in the main stalk (C), in the transitional zone between the cilium and the basal body (D), and in the basal body (E). (Reproduced from *American Review of Respiratory Diseases*, v. 93, no.3 part 2, page 12)

Figure 4



A "turner" embedded in a mass of other tissue. (magnification, x 430
Photograph by Melvin Hinton, Durham, North Carolina)

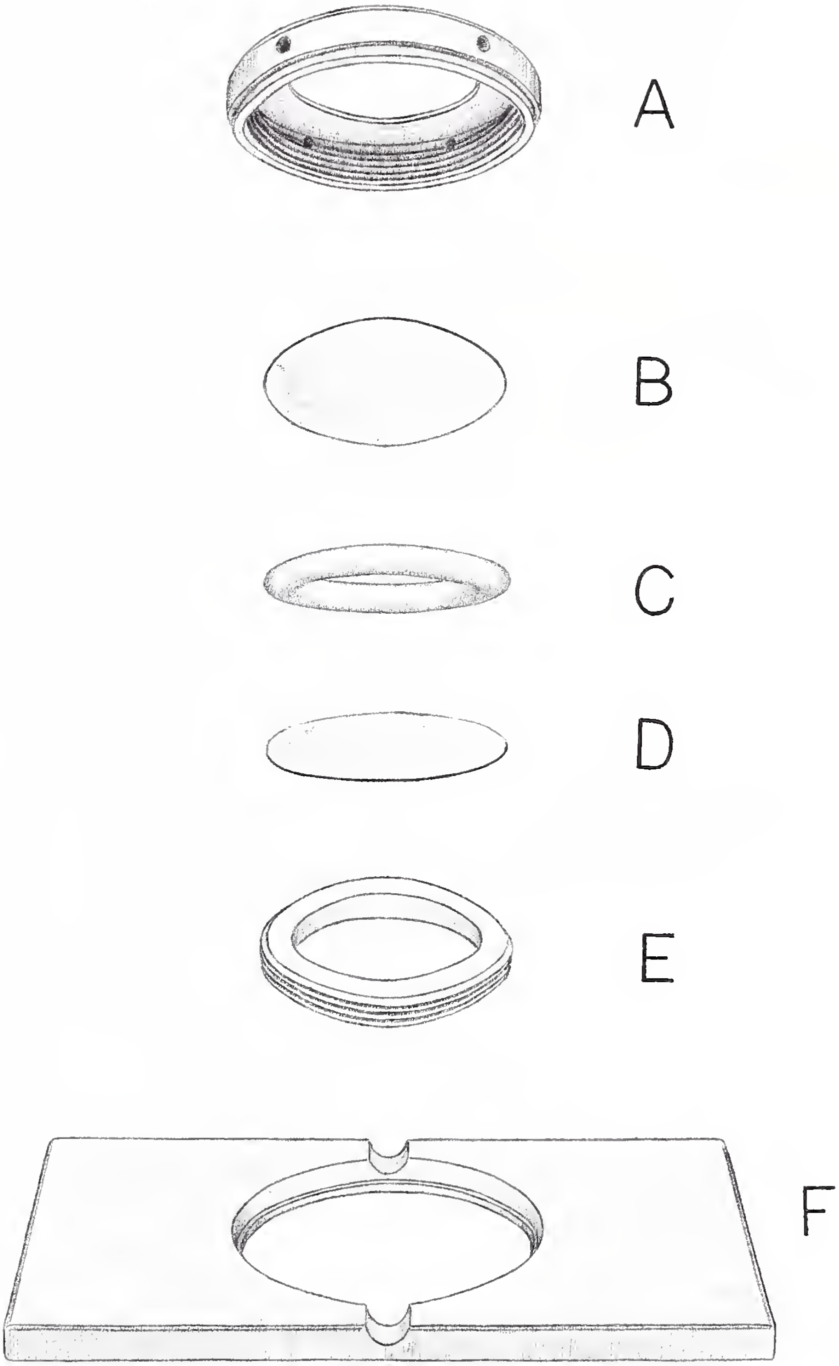
Figure 5



Rose Chamber

(Reproduced from *American Review of Respiratory Diseases*, v.93, No. 3
part 2, page 62)

Figure 6.

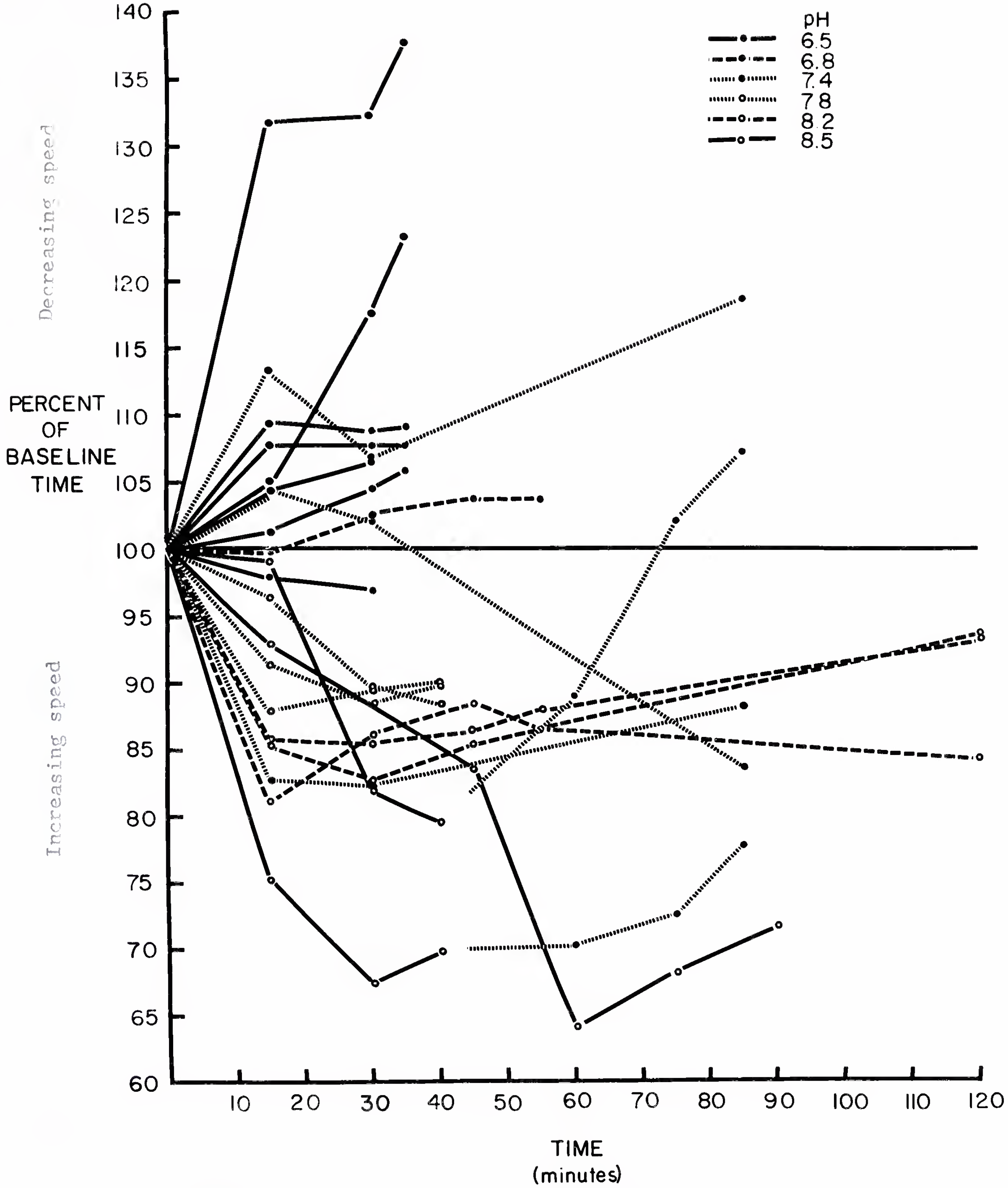


Sykes-Moore
Tissue Culture
Chamber

Figure 6: Sykes-Moore Tissue Culture Chamber showing its component parts: A--metal top of the chamber
B & D--25mm diameter coverslips
C--silicone rubber gasket
E--metal bottom of the chamber
F--chamber holder

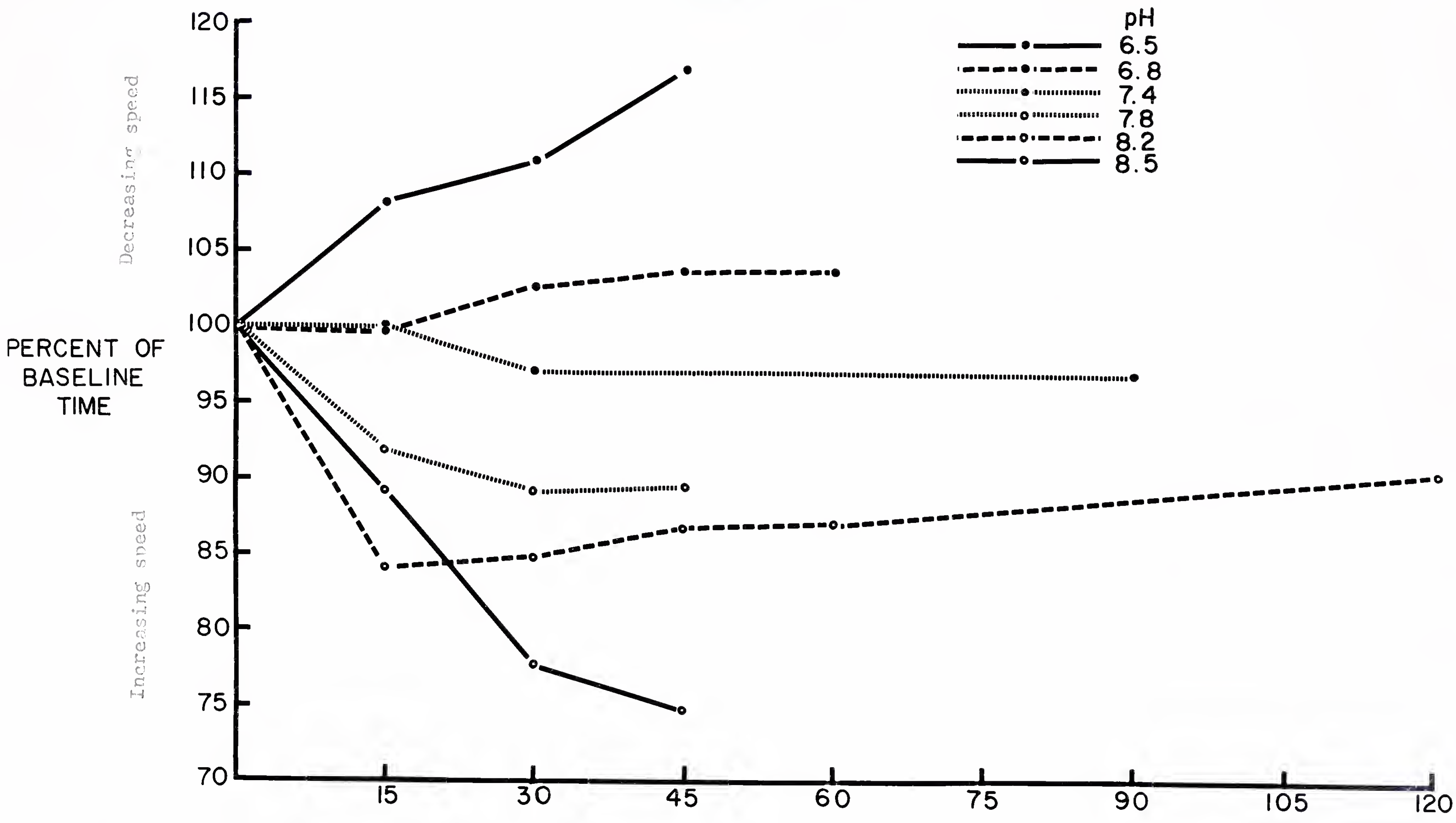
(Drawing by B. MacAnally)

Figure 7



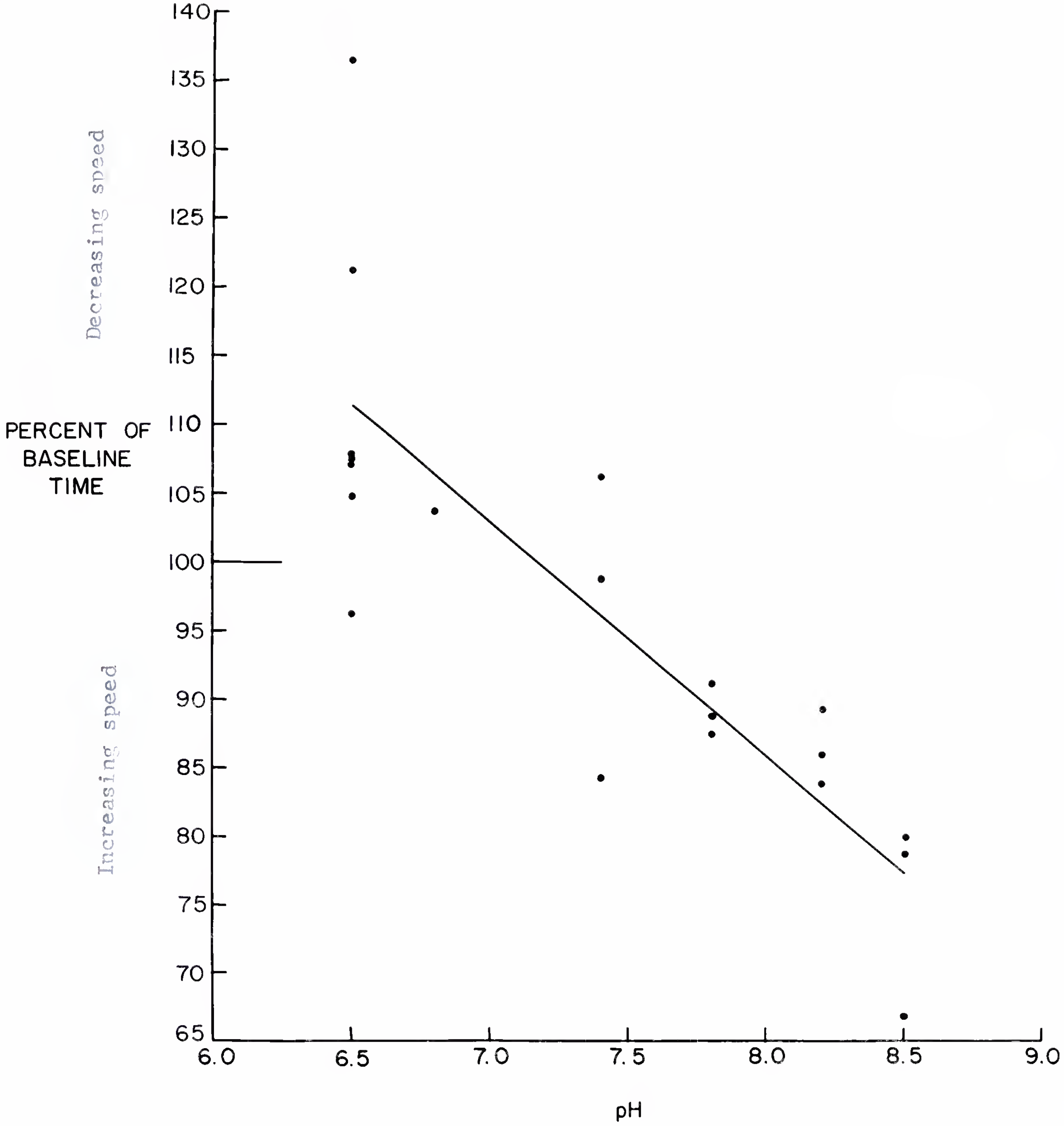
Percent of baseline time for 10 rotations vs. time after addition of test solution. Each line represents one "turner". Two "turners" were initially perfused with a test solution of pH 8.5 and then reperfused with a solution of pH 7.2 as indicated on the graph. These reperfusions are indicated on the graph with pH 7.4 lines although the reperfusion was actually done at pH 7.2.

Figure 8



Percent of baseline time for 10 rotations vs. time after addition of test solution. Each line represents the average values for all the "turners" at each pH.

Figure 9



Percent of baseline time for 10 rotations vs. pH of the test solution. The points on the graph are the percent of baseline time for each "turner" at time= 30 minutes. The line is a regression line with a slope of -16.81, r=0.84 and t(18 degrees of freedom) greater than 6, $p < .01$.

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